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Onsekiz Uçucu Yağın Bazı Maya ve Bakterilere Karşı Minimum İnhibitör Konsantrasyonunun Resazurin Yöntemi ile Belirlenmesi

The Determination of Minimum Inhibitory Concentration of Eighteen Essential Oils with Resazurin method, Against a Group of Yeast and Bacteria

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Öz

GİRİŞ ve AMAÇ: Bu çalışmanın amacı, insan ve hayvanlar açısından önemli bazı patojenik bakteri ve mantarlara karşı, hidrodistillasyon yöntemi ile elde edilen ve GC-MS yöntemi aile ayrıştırılan 18 çeşit uçucu yağın minimum inhibitör konsantrasyon değerlerini araştırmaktır.
YÖNTEM ve GEREÇLER: Achillea gypsicola (Asteraceae), Ruta graveolens L. (Rutaceae), Satureja hortensis L. (Lamiaceae), Thymbra spicata L. (Lamiaceae), Achillea biebersteinii Afan. (Asteraceae), Artemisia santonicum L. (Asteraceae), Foeniculum vulgare. (Umbelliferae), Origanum acutidens (Lamiaceae), Thymus fallax (Lamiaceae), Inula graveolens L. (Asteraceae), Dorystoechas hastata (Lamiaceae), Crambe orientalis L. (Brassicaceae), Narenciye cinensis L. (Rutaceae), Satureja spicigera (Lamiaceae), Lavandula stoechas L. (Lamiaceae), Satureja montana L. (Lamiaceae), Vitex agnus-castus L. (Verbenaceae), Origanum majorana L. (Lamiaceae).
BULGULAR: Çalışmamızda yağlar dört bakteri ve üç mantara karşı test edildi; Acinetobacter baumanii ATCC 49139, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 29213, E. coli ATCC 25923, Candida albicans ATCC 14053, Candida glabrata ATCC 15126, Candida parapsilosis ATCC 22019 mikroorganizmalarının yağlara karşı minimum inhibitör konsantrasyonu bulundu. A. gypsicoala, R. graveolens, S. hortensis, T. spicata, O. majorana ve V. agnus iki dilüsyon tekrarında da bulunan en etkili yağlardır.
TARTIŞMA ve SONUÇ: Çalışmamız, bu yağların hem insan hem de hayvan yaralarının antibakteriyel ve antifungal tedavisine ek olarak kullanılmasının faydalı olacağı düşüncesiyle gerçekleştirilmiştir.

Anahtar Kelimeler: Aşı, Çocuk, Hepatit A, Seroprevalans

Abstract

INTRODUCTION: Aim of this study is investigate the effect and MIC values of some essential oils, purified by hydrodistillation method, in some of the pathogenic bacteria and fungi.
METHODS: Achillea gypsicola Hub. (Asteraceae), Ruta graveolens L. (Rutaceae), Satureja hortensis L. (Lamiaceae), Thymbra spicata L. (Lamiaceae), Achillea biebersteinii Afan. (Asteraceae), Artemisia santonicum L. (Asteraceae), Foeniculum vulgare Mill. (Umbelliferae), Origanum acutidens (Hand-Mazz.) (Lamiaceae), Thymus fallax Fisch. & C.A. Mey. (Lamiaceae), Inula graveolens L. (Asteraceae), Dorystoechas hastata Boiss. & Heldr. Ex Bentham (Lamiaceae), Crambe orientalis L. (Brassicaceae), Citrus cinensis L. (Rutaceae), Satureja spicigera (C. Koch) Boiss. (Lamiaceae), Lavandula stoechas L. (Lamiaceae), Satureja montana L. (Lamiaceae), Vitex agnus-castus L. (Verbenaceae), Origanum majorana L. (Lamiaceae) (Rutaceae).
RESULTS: In our study oils were tested against four bacteria and three fungi. The minimum inhibitory concentration and minimum bactericidal concentration values of oils on Acinetobacter baumanii ATCC 49139, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 29213, Eescherichia coli ATCC 25923, Candida albicans ATCC 14053, Candida glabrata ATCC 15126, Candida parapsilosis ATCC 22019 microorganisms was found.
DISCUSSION and CONCLUSION: It is aimed to find MIC values of oils by adding resazurin dye as a redox indicator.

Keywords: Bacteria and Yeasts, Essential oils, MIC, REMA

INTRODUCTION

Its known that essential oils or volatile oils have antibacterial, antifungal, anti carcinogenic and anti stress effects on body (1-3). Antimicrobial effects of oils have been used in alternative medicine since ancient times (4). Essential oils can be purified by methods such as steam distillation, hydrodiffusion, hydrodistilation or solvent extraction (5). Antimicrobial activity of

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volatile oils presents an increasing interest in the recent years and there are studies about the effectiveness of oils on multidrug resistant strains (6). There are studies that focused on use of essential oils in cosmetic, food and pharmaceutical industry too. Many studies in the last years focused on the beneficial properties of the essential oils, including antibacterial and antifungal properties, research field of essential oils has a wide range (7).

Common use of unnecessary antibiotics to organisms resulted with high resistance against them. However, essential oils are antimicrobial agents that can be used to prevent bacterial resistance in drug unnecessary cases (8). Essential oils are natural treatment agents in the past and probably in the future so investigating the antimicrobial activity of essential oils are important for the future treatments (9). A large part of the population in our country who cannot access or choose modern medicine uses natural treatment methods (10).

Some methods are used for detecting the antimicrobial effects of oils. The techniques used in the determination of antimicrobial activity are examined under two headings; as diffusion and dilution methods. MIC values of essential oils can be determined by dilution method. Disk diffusion is the most commonly used method for determining the antimicrobial activity of essential oils although this method is suitable for determining the presence of antimicrobial activity of essential oils, it is not suitable for comparing the results with published data (11).

The microdilution method is easy to carry out, standardized and inexpensive.

Therefore, it is more effective using of the microdilution method (12).

Aim of this study is investigate the effect and MIC values of some essential oils purified by hydrodistillation method, in some of the pathogenic bacteria and fungi. Achillea gypsicola Hub. (Asteraceae), Ruta graveolens L. (Rutaceae), Satureja hortensis L. (Lamiaceae), Thymbra spicata L. (Lamiaceae), Achillea biebersteinii Afan. (Asteraceae), Artemisia santonicum L. (Asteraceae), Foeniculum vulgare Mill. (Umbelliferae), Origanum acutidens (Hand-Mazz.) (Lamiaceae), Thymus fallax Fisch. & C.A. Mey. (Lamiaceae), Inula graveolens L. (Asteraceae), Dorystoechas hastata Boiss. & Heldr. Ex Bentham (Lamiaceae), Crambe orientalis L. (Brassicaceae), Citrus cinensis L. (Rutaceae), Satureja spicigera (C. Koch) Boiss. (Lamiaceae), Lavandula stoechas L. (Lamiaceae), Satureja montana L. (Lamiaceae), Vitex agnus-castus L. (Verbenaceae), Origanum majorana L. (Lamiaceae) (Rutaceae) oils were tested against four bacteria and three fungi; Acinetobacter baumanii ATCC 49139, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 29213, E. coli ATCC 25923, Candida albicans ATCC 14053, Candida glabrata ATCC 15126, Candida parapsilosis ATCC 22019 microorganisms, minimum inhibitory concentration, minimum bactericidal concentration was found. It is aimed to find MIC values of oils by adding resazurin dye as a redox indicator.

METHODS

Plant Material and Isolation of Essential Oils

Flowering stages of A. gypsicola, R. graveolens, S. hortensis, T spicata, A. biebersteinii, A. santonicum, F. vulgare, O. acutidens, T. fallax, I. graveolens, D. hastata, C. orientalis, C. cinensis, S. spicigera, L. stoechas, S. montana, V. agnus, O. majorana were collected from different localities in Turkey between June 2017 and August 2019. Then 500 g was hydrodistilled for 4 h using a Clevenger-type apparatus. Hydrodistillation of 18 oils yielded 0.9, 0.8, 1.1, 0.7, 1.5, 0.5, 0.6, 0.5, 1.4, 1.7, 1.9, 0.6, 2.1, 1.9, 0.8, 2.2, 2.1 and 1.5% (w/w) of plant essential oil based on dried parts of the tested plants, respectively. The drying of the plant essential oils was done with anhydrous Na2SO4. The plant essential oils were stored in a

freezer at 4°C during testing.

Gas Chromotography/mass Spectrometry (GC and GC/MS Analysis)

Gas chromatography/mass spectrometry (GC/MS) method is gold standard for analysis of oil spill samples correlation analysis. GC and GC/MS analyzes of essential oil were prepared using the literatüre (13-28)

Test Microorganisms

Oils were tested against four bacteria and three fungi; Acinetobacter baumanii ATCC 49139, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 29213, E. coli ATCC 25923, Candida albicans ATCC 14053, Candida glabrata ATCC 15126, Candida parapsilosis ATCC 22019 microorganisms.

Microdillussion Procedure

Preparation of yeast and bacteria. The yeasts to be studied are set at 0.5 McFarland. Oil preparation. 1000 µl of ethanol, 10 µl of each oil is placed, vortexed. Preparation of dilutions. 1/1000 stok solutions are prepared from yeast cultures. 1/20 stok solutions are prepared for bacterial cultures. Preparation of microplates. 100 µl of medium added in each well, 100 µl of oil stock solution added into the first well. 100 µl are diluted with serial dilutions. 100 µl of yeast suspension added at last in each well. Incubated at 37°C for 48 h. After 48 hour resazurin dropped.

4. Finding MIC Values with Resazurin

0.001 g of resazurin dissolve in 10 ml of sterile distilled water and filtered. It can be stored at +4 °C degrees for 1 week and the value is determined by adding 10 microliters of resazurin prepared to each well. Active bacterial cells reduce the non-fluorescent resazurin (blue) to the fluorescent resorufin (pink) which can be further reduced to hydroresorufin.

RESULTS

Chemical composition of the plants are found with GC/MS method and given in the table 1 with literature.

DISCUSSION

Essential oils are known to contain some components that inhibit the metabolic activities of bacteria, molds and yeasts. Their antimicrobial activities are based on phenolic terpenoid components (thymol, carvacrol, eugenol), aldehydes and organic acids (29). Over 1500 or more plants have been reported to have antimicrobial effects in the literature. As doses that provide the desired antimicrobial effect exceed sensory acceptable limits, their use as a preservative in foods or their use in treatment are limited (30). For this reason, studies to determine the amount of essential oils minimum inhibitory concentration is very important they will be treatment choice for the clinicians in the the future.

Resazurin is one of the effective methods in diagnosing the minimum inhibitory concentration due to the bacteria's ability to metabolize resazurin at such high concentrations (31).

It has been determined that some essential oils should have various restrictions on their use. For example, undiluted essential oil produced a phototoxic effect in mice and pigs. It has been reported that after ingestion of 15 g of essential oil in adults, convulsions, cramping of the jaw muscles and foaming in the mouth develop and disappear within 48 hours (32-34). Therefore, it is important to determine the amount of minimum inhibitör concentration of essential oils to avoid problems such as overdose, in traditional treatments. Hong et all. use Pinus densiflora, Pinus koraiensis and Chamaecyparis obtusa against Salmonella typhimurium, Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonisa and Candida

albicans with disk diffusion method in different concentrations and they found that P. densiflora and C. obtusa have antibacterial effects, P. koraiensis and C. obtusa have antifungal effects (35).

Sai''dana et all. use Tamarix boveana oil against six gram positive and gram negatice bacteria and four fungi; S. aureus, E. coli, Pseudomonas aeruginosa, Micrococcus lutens, S. typhimurium, Fusarium oxysporum, Aspergillus niger. T. boveana volatile oils exhibited an interesting antibacterial activity except P. aeruginosa no antifungal activity was detected with this oil (36).

Rota et all. use Thymus vulgaris, Salvia sclarea, Salvia officinalis, Lavandula angustifolia, Rosemarinus officinalis, Satureja montana against S. typhimurium, Salmonella enteridis, Shigella flexneri, L. monocytogenes, S. aureus, E. coli. esc. And they found S. montana and T. vulgaris were the most inhibitory oils examined. The MIC was lower for the gram-positive bacteria (L. monocytogenes and S. aureus) than for the gram-negative bacteria (Salmonella enteritidis, S. typhimurium, E. coli O157:H7, Yersinia enterocolitica, and S. flexneri) (37).

Looking at the data we have, it is possible to say that A. gypsicoala, R. graveolens, S. hortensis, T. spicata, O. majorana and V. agnus are the most effective oils against; E. coli, P. aeruginosa and C. albicans. F. vulgare, C. orientalis, C. cinensis, S. spicigera, L. stoechas, S. montana in less effective oils on E. coli, P. aeruginosa and C. albicans. While the most effective oil for yeast species except for C. parapsilosis is found as O. majorana, the effectiveness of this oil against C. parapsilosis has not been determined. Again, among the 18 oils in our study, there is no oil effective against A. baumanii and S. aureus. C. glabrata showed the highest resistance to whole oils (found resistant to; F. vulgare, O. acutidens, T. fallax, I. graveolens, D. hastata, C. orientalis, C. cinensis, S. spicigera).

Conclusion

The use of traditional oils in treatments is a known fact for centuries. In our study, we tried some of the oils used for therapeutic purposes in our country against bacteria that have high pathogenic properties in humans and animals. It seems A. gypsicoala, R. graveolens, S. hortensis, T. spicata, O. majorana and V. Agnus oils are very promising for the treatment of the E. coli, P. aeruginosa and C. albicans. They can be use novel therapeutic strategies. When these results are evaluated, it has been revealed that these species may be among the potential biological materials that may assist or alternative to antibiotics or antifungals after the necessary tests have been carried out.

Special thanks to the identification of plant materials was made by XX, department of biology, faculty of art and science, XX University. The voucher specimens of these plants have been deposited in the herbarium of XX University. Aerial parts of the plants were dried in shade before processing with a grinder and XX from XX University XX department.

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REFERENCES

1. Michalak M. The use of carrier oils in aromatherapy massage and their effect on skin. Archives of Physiotherapy & Global Researches. 2018; 22:23-31.
2. Vostinaru O, Heghes SC, Filip L. Safety Profile of Essential Oils. In Essential Oils-Bioactive Compounds, New Perspectives and Applications. IntechOpen. 2020. https://www.intechopen.com/books/essential-oils-bioactive-compounds-new-perspectives-and-applications/safetyprofile-of-essential-oils.05.10.2020.
3. Xu Y, Chengyuan L, Hailing F, Xiwu Q, Weilin L, Qiungwen S. Variation of trichome morphology and essential oil composition of seven Mentha species.

Biochemical Systematics and Ecology 2018; 79:30-36.
4. Bahman N, Fatemeh J. Analysis of the Essential Oil from Mentha pulegium and Identification of its Antioxidant Constituents. Journal of Essential Oil Bearing Plants 2018; 21:223-229.
5. Abdeli M, Moghrani H, Aboun A, Maachi R, Algerian Mentha pulegium L leaves essential oil: Chemical composition, antimicrobial, insecticidal and antioxidant activities. Industrial Crops and Products 2016; 94:197-205.
6. Ali B, Al-Wabel NA, Shams S, Ahamad A, Khan SA, Anwar F. Essential oils used in aromatherapy: A systemic review. Asian Pac J Trop Biomed. 2015; 5:589-598
7. Chawla S, Thakur M Overview of mint (Mentha L.) as a promising health-promoting herb. International Journal of Pharmaceutical Research & Development 2013; 5:073-080.
8. Chairgulprasert V, Prasertsongskun S, Wichaporn W. Chemical constituents of the essential oil and antibacterial activity of Zingiber wrayi var. halabala Songklanakarin. J Sci Tech 2005; 27:8-813.
9. Khaki A, Badie Davatgar A, Nikniaz H, Farnam A. Treatment Effects of Onion (Allium cepa) and Ginger (Zingiber officinale) on Sexual Behavior of Rat after Inducing an Antiepileptic Drug (lamotrigine). Balkan Med J 2012; 29:236-242.
10. Lambert RJW, Pearson, J. Susceptibility testing: accurate and reproducible minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values. Journal of applied microbiology 2000; 88:784-790.
11. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils–a review. Food and chemical toxicology 2008; 46:446-475.
12. Dorman HJD, Deans, SG. Antimicrobial agents from plants, antibacterial activity of plant volatile oils. Journal of Applied Microbiology 2000; 88:308-316.
13. Amor G, Caputo L, La Storia, De Feo A, V, Mauriello, G, Fechtali, T. Chemical Composition and Antimicrobial Activity of Artemisia herba-alba and Origanum majorana Essential Oils from Morocco. Molecules 2019; 24:4021.
14. Chaaban SB, Hamdi, SH, Mahjoubi K, Jemâa, JMB. Composition and insecticidal activity of essential oil from Ruta graveolens, Mentha pulegium and Ocimum basilicum against Ectomyelois ceratoniae Zeller and Ephestia kuehniella Zeller (Lepidoptera: Pyralidae). Journal of Plant Diseases and Protection 2019; 126:237-246.
15. Ferrante C, Zengin, G, Menghini L, Diuzheva A, Jekő J, Cziáky Z, Lobine D. Qualitative Fingerprint Analysis and Multidirectional Assessment of Different Crude Extracts and Essential Oil from Wild Artemisia santonicum L. Processes 2019; 7:522.
16. Vieira JN, Gonçalves CL, Villarreal JPV, Gonçalves VM, Lund RG, Freitag RA, Nascente PS Chemical composition of essential oils from the apiaceae family, cytotoxicity, and their antifungal activity in vitro against candida species from oral cavity. Brazilian Journal of Biology 2019; 79:432-437.
17. Khavarpour M, Vahdat SM, Moghadamnia AA, Hasanzadeh O, Salimi Z, Rahmanpour N. Chemical Composition, Antibacterial and Analgesic Activity of Lavandula stoechas Flowers from North of Iran. International Journal of Engineering 2019; 32:1065-1073.
18. Kirkan B, Sarikurkcu C, Amarowicz R. Composition, and antioxidant and enzyme-inhibition activities, of essential oils from Satureja thymbra and Thymbra spicata var. spicata. Flavour and Fragrance Journal 2019; 34:436-442.
19. Rezaei M, Razmjoo J, Ehtemam MH, Karimmojeni H, Zahedi M. The interaction between shade and drought affects essential oil quantity and quality of Vitex agnus-castus L. leaves and seeds. Industrial Crops and Products 2019; 137:460-467.
20. Karan T, Yildiz I, Aydin A, Erenler, R. Inhibition of Various Cancer Cells Proliferation of Bornyl Acetate and Essential Oil from Inula graveolens (Linnaeus) Desf. Records of Natural Products 2018; 12:273-83.
21. Usanmaz-Bozhuyuk A, Kordali Ş Investigation of the toxicity of essential oils obtained from six Satureja species on Colorado Potato Beetle, Leptinotarsa decemlineata. Fresen Environ Bull 2018; 27:4389-4401.
22. Çakır A, Özer H, Aydın T, Kordali Ş, Çavuşoglu AT, Akçin T. Phytotoxic and Insecticidal Properties of Essential Oils and Extracts of Four Achillea Species. Records of Natural Products 2016; 10: 154-167.
23. Kan A, Günhan RS, Çelik SA. The Chemical Composition Profile of Dorystoechas hastata Boiss. & Heldr. Ex Bentham Cultivated in Turkey. Rec. Nat. Prod 2015; 9:1, 135-145.
24. Gulec AK, Erecevit P, Yuce E, Arslan A, Bagci E, Kirbag, S. Antimicrobial Activity of the Methanol Extracts and Essential Oil with the Composition of Endemic Origanum acutidens (Lamiaceae). Journal of Essential Oil Bearing Plants 2014; 17:353-358.
25. Kotan R, Cakir A, Dadasoglu F, Aydin T, Cakmakci R, Ozer H, Dikbas N. Antibacterial activities of essential oils and extracts of Turkish Achillea, Satureja and Thymus species against plant pathogenic bacteria. Journal of the Science of Food and Agriculture 2010; 90:145-160.
26. Kordali S, Cakir A, Akcin TA, Mete E, Akcin A, Aydin T, Kilic, H. Antifungal and herbicidal properties of essential oils and n-hexane extracts of Achillea gypsicola Hub-Mor. and Achillea biebersteinii Afan. (Asteraceae). Industrial crops and products 2009; 29:562-570.

27. Razavi, S M, Nejad-Ebrahimi S. Chemical composition, allelopatic and cytotoxic effects of essential oils of flowering tops and leaves of Crambe orientalis L. from Iran. Natural product research 2009; 23:1492-1498.
28. Kirbaslar FG, Kirbaslar SI, Pozan G, Boz I. Volatile constituents of Turkish orange (Citrus sinensis (L.) Osbeck) peel oils. Journal of Essential Oil Bearing Plants 2009; 12:586-604.
29. Baser KHC, Özek T, Kırımer N, Tümen GA comparative study of the essential oils of wild and cultivated Satureja hortensis. Journal of Essential Oil Research 2004; 16:422-424.
30. Cutter CN. Antimicrobial effect of herb extracts against Eshericia coli O157:H7, Listeria monocytogenes, and Salmonella Typhimurium associated with beef. Journal of Food Protection, 2000; 63:601-7.
31. Singh CR, Kathiresan K, Anandhan S, Suganthi K. Antioxidant and antibacterial activity of field grown and tissue cultured root callus of mangrove species. Eur J Med Plants 2014; 11:723–742.
32. Kaya GI. Artemisia absinthium, FFD Monografları Tedavide Kullanılan Bitkiler. Ed. Demirezer Ö. Medikal & Novel Tıp Kitapsarayı Ankara. 2011; 83-87.
33. ESCOP Monographs. Absinthii Herba (2nd ed.) Thieme. New York NY 2003;3-7.
34. Höld KM, Sirisoma NS, Ikeda T. α-Thujone (the active component of absinthe): γ-aminobutyricacid type A receptor modulation and metabolic detoxification. Proc Natl Acad Sci USA 2000; 97:3826-31.
35. EJ Hong, KJ Na, IG Choi, KC Choi, EB Jeung. Antibacterial and antifungal effects of essential oils from coniferous trees Biol. Pharm. Bull 2004; 27: 863-866.
36. Saı¨dana, D, Mahjoub, MA, Boussaada, O, Chriaa, J, Chéraif, I, Daami, M, Mighri, Z, Helal, AN. Chemical composition and antimicrobial activity of volatile compounds of Tamarix boveana. Microbiol Res 2008; 163:445-55.
37. Rota C, Carraminana JJ, Burillo J, Herrera A. In vitro antimicrobial activity of essential oils from aromatic plants against selected foodborne pathogens J. Food Prot 2004; 67:1252-1256.

Tablo 1. Chemical composition of essential oils

Plant	Composition	%	References
Achillea biebersteinii Afan. (Asteraceae)	1,8-Cineole	38.1	Çakır et al.[22]
	Camphor	23.6	
	Borneol	5.9	
	α-Terpineol	5.2	
Achillea gypsicola Hub. (Asteraceae)	1,8-Cineole	22.01	Kordali et al.[26]
	Camphor	40.17	
	Piperitone	11.29	
	Borneol	9.50	
Artemisia santonicum L. (Asteraceae)	Camphor	36.6	Ferrante et al.[15]
	1,8-Cineole	10.2	
	α-thujone	10.1	
	Borneol	4.5	
Crambe orientalis L. (Brassicaceae)	2-Methyl-5-hexenenitrile	19.5	Razavi et al.[27]
	3-Butenylisothiocyanate	15.0	
	Benzyl cyanide	16.9	
	Octyl octanoae	4.8	
Citrus cinensis L. (Rutaceae)	Limonene	91.14	Kirbaslar et al.[28]
	Myrcene	1.30	
	Octanal	1.38	
	Sabinene	0.95	
Dorystoechas hastata Boiss. & Heldebr. Ex Bentham (Lamiaceae)	1,8-Cineole	16.6	Kan et al.[23]
	Borneol	15.0	
	β-pinene	9.7	
	α-pinene	8.3	
Foeniculum vulgare Mill. (Umbelliferae)	Trans-dihydrocarvone	79.62	Vieira et al.[16]
	Anethole	12.19	
	Fenchone	3.65	
	Estragole cis-anethole	2.89	
	Methyl nonadecanoate		
Inula graveolens L. (Asteraceae)	Bornyl acetate	60.43	Karan et al.[20]
	Caryophyllen oxide	11.58	
	Borneol	11.34	
	δ-Cadinol	4.89	
Lavandula stoechas L. (Lamiaceae)	Linalol	35.69	Khavarpour et al.[17]
	Borneol	14.99	
	1,8-Cineole	11.45	
	Camphor	4.32	
Origanum acutidens (Hand-Mazz.) (Lamiaceae)	Carvacrol	61.8	Gulec et al.[24]
	p-cymene	15.5	
	γ-terpinene	1.4	
	Borneol	1.2	
Origanum majorana L. (Lamiaceae)	Terpinen-4-ol	34.1	Amor et al.[13]
	α-Terpinene	19.2	
	endo-Fenchyl-acetate	9.8	
	Terpineol	8.9	
Ruta graveolens L. (Rutaceae)	1-nonene	19.2	Chaaban et al. [14]
	2-undecanone	16.2	
	1-Pentadecene	12.6	
	2-nonanone	11.9	
Satureja hortensis L. (Lamiaceae)	Thymol	72.18	Usanmaz Bozhuyuk et al.[21]
	p-cymene	9.74	
	γ-terpinene	7.61	
	Carvacrol	7.29	
Satureja spicigera (C.Koch) Boiss. (Lamiaceae)	Carvacrol	90.25	Usanmaz Bozhuyuk et al.[21]
	p-cymene	4.12	
	γ-terpinene	2.58	
	β-bisabolene	1.38	

Satureja montana L. (Lamiaceae)	Carvacrol	71.31	Usanmaz Bozhuyuk et al.[21]
	γ-terpinene	11.87	
	p-cymene	6.06	
	β-Caryophyllene	4.70	
Thymus fallax Fisch. & C.A. Mey. (Lamiaceae)	Carvacrol	51.26	Kotan et al.[25]
	β-Caryophyllene	5.25	
	γ-Cadinene	5.13	
	1,8-Cineole	5.12	
Thymbra spicata L. (Lamiaceae)	Carvacrol	63.23	Kirkan et al. [18]
	γ-Terpinene	18.94	
	p-Cymene	8.31	
	α-Terpinene	2.45	
Vitex agnus-castus L. (Verbenaceae)	α-pinene	39.44	Rezaei et al.[19]
	β-Terpinyl acetate	30.62	
	Caryophyllene	8.96	
	β-Eudesmene	4.74	

Table 2. MIC values of microorganisms

Minimal Inhibitory Concentrations of Microorganisms (µg/ml)*							
Oils**	E.coli	A.baumanii	S.aureus	P.aeruginosa	C.albicans	C.glabrata	C.paraphysilosis
1	1.56	0.78	0.39	6.25	0.625	0.039	0.039
2	1.56	0.78	0.19	6.25	0.312	0.039	0.039
3	1.56	0.39	0.39	1.56	0.312	0.019	0.019
4	1.56	0.39	0.39	1.56	0.312	0.019	0.019
5	1.56	0.39	0.19	1.56	0.156	0.039	0.019
6	0.19	0.04	0.19	1.56	0.312	0.078	0.039
7	0.19	0.78	0.19	1.56	0.312	R	0.039
8	0.19	0.09	0.09	6.25	0.312	R	0.039
9	0.19	0.78	0.78	6.25	0.156	R	0.0097
10	0.19	0.39	0.19	6.25	0.156	R	0.019
11	0.19	0.39	0.78	6.25	0.156	R	0.019
12	1.56	0.78	0.78	6.25	0.156	R	0.0097
13	1.56	0.78	0.78	6.25	0.078	R	R
14	1.56	0.39	0.39	6.25	0.156	R	R
15	1.56	0.78	0.78	6.25	0.078	0.156	0.0097
16	1.56	0.19	0.78	6.25	0.156	0.078	0.0097
17	3.12	0.78	R	3.12	1.25	0.156	0.019
18	6.25	0.78	0.04	3.12	1.25	0.312	0.019

*Yeast dilusions: 1.well 10 µg/ml, 2. .well 5 µg/ml, 3 .well 2.5 µg/ml, 4 .well 1.25 µg/ml, 5 .well 0.625 µg/ml, 6 .well 0.312 µg/ml, 7.well 0.156 µg/ml, 8 .well 0.078 µg/ml, 9 .well 0.039 µg/ml, 10 .well 0.019 µg/ml, 11 .well 0.0097 µg/ml, 12.well 0.0048 µg/ml. Bacterial dilusions: 1.well 50 µg/ml, 2. .well 25 µg/ml, 3 .well 12.5 µg/ml, 4 .well 6.25 µg/ml, 5 .well 3.125 µg/ml, 6.well 1.56 µg/ml, 7.well 0.78 µg/ml, 8.well 0.39 µg/ml, 9 .well 0.19 µg/ml, 10 .well 0.09 µg/ml, 11 .well 0.04 µg/ml, 12 .well 0.02 µg/ml.

**1-A.gypsicoala, 2-R. graveolens, 3-S. hortensis,4- T spicata, 5-A. biebersteinii ,6- A. santonicum, 7-F.vulgare,8- O. acutidens , 9-T. fallax,10- I. graveolens, 11-D. hastata,12- C. orientalis , 13-C. cinensis, 14-S.spicigera, 15-L. stoechas, 16-S. montana, 17-V. agnus, 18- O. majorana.

A. gypsicoala, R. graveolens, S. hortensis, T. spicata, O. majorana and V. agnus are the most effective oil. O. majorana was determined to be the most effective essential oil except for C. parapsilosis. There is no oil effective against A. baumanii and S. aureus. C. glabrata showed the highest resistance to whole oils. Figure 1 shows resazurin based color difference.

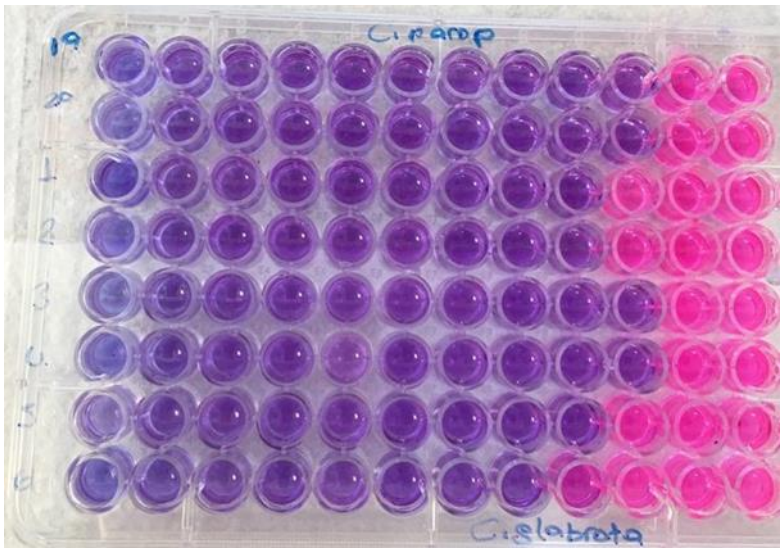

Figure 1.Resazurin based color difference.